4500
FS-SO-2203-1.33
(Problem 2)

Study Plan Summary
S. J. Barras and J. Brand-Pineville, La.
A cooperative agreement with University of Iowa

INFLUENCE OF MICROORGANISMS ON THE PHEROMONAL REGIME OF SOUTHERN PINE BEETLE

The object of this cooperative study is to investigate the production by microorganisms of a number of compounds known (or thought) to elicite behavioral responses in D. frontalis and/or important parasites or predators. Bacteria, yeasts, and fungi associated with the southern pine beetle will be tested for their ability to transform or synthesize a variety of chemicals (e.g. α-pinene, β-pinene, myrcene, trans-verbenol, verbenone, myrtenol, frontalin). This will be done by, (1) growing organisms on a medium containing a candidate chemical, (2) adding the candidate substrate after growth has occurred, (3) adding the substrate to either whole cell suspensions or cell-free preparations, (4) growing organisms on phloem.

Resultant chemicals will be purified by preparative gas chromatography, and mass spectra of isolated compounds will be compared to standards. When necessary, IR, NMR, and UV spectra will also be obtained.

Resulting chemical products showing promise as behavioral chemicals will be field bioassayed alone or in combination with other known behavioral chemicals. In addition initially unknown compounds will be tested to determine their attractiveness to the beetle or parasites and predators.

UNITED STATES DEPARTMENT OF AGRICULTURE

FOREST SERVICE

4500
FS-SO-2203-1.33
(Problem 2)

STUDY PLAN

INFLUENCE OF MICROORGANISMS ON THE PHEROMONAL REGIME OF SOUTHERN PINE BEETLE

(A cooperative agreement with University of Iowa and Southern Forest Experiment Station)

by

Dr. John Brand
Department of Microbiology
University of Iowa
Iowa City, Iowa 52242

Dr. Stanley J. Barras
Southern Forest Experiment Sta.
Forest Insect Research
Pineville, LA 71360

RWU 2203

Southern Forest Experiment Station

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FS-SO-2203-1.33 (Problem 2) — "Influence of Microorganisms on the Pheromonal Regime of Southern Pine Beetle"

(STUDY NUMBER) (TITLE)

Stanley J. Barras

(AUTHOR SIGNATURE) Stanley J. Barras, Acting Project Leader (DATE) 1/30/75

REMARKS:

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FS-SO-2203-1.33 (Problem 2) (STUDY NUMBER)

"Influence of Microorganisms on the Pheromonal Regime of Southern Pine Beetle" (TITLE)

Stanley J. Barras (AUTHOR SIGNATURE)

Stanley J. Barras, Acting Project Leader (DATE) 1/30/75

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(Problem 2)

STUDY PLAN

INFLUENCE OF MICROORGANISMS ON THE PHEROMONAL REGIME OF SOUTHERN PINE BEETLE

Cooperative Agreement #19-205 with University of Iowa

Prepared by:

J M Brand — 23 June 1975
John Brand (Date)
Department of Microbiology
University of Iowa

Stanley J. Barras — 6/25/75
Stanley J. Barras (Date)
Project Leader
Forest Insect Research

Approved by:

Stanley J Barras — 6/25/75
Stanley J. Barras (Date)
Project Leader
RWU 2203

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FS-SO-2203-1.33

STUDY PLAN

INFLUENCE OF MICROORGANISMS ON THE PHEROMONAL REGIME OF SOUTHERN PINE BEETLE

Cooperative Agreement Number USDA-SFES 19- 205

Dr. John Brand

College of Medicine

Department of Microbiology

University of Iowa

Iowa City, Iowa 52242

and

Dr. Stanley J. Barras

Southern Forest Experiment Station

Forest Insect Research

Pineville, LA 71360

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(Problem 2)

STUDY PLAN

INFLUENCE OF MICROORGANISMS ON THE PHEROMONAL REGIME OF SOUTHERN PINE BEETLE

INTRODUCTION

Various chemical compounds are associated with bark beetles and are known to act as aggregation pheromones and/or sex attractants. These substances occur in the hindgut and are passed out in the feces or possibly directly as volatiles. However, the actual site or means of production in the insect have not been determined for most species including the southern pine beetle (SPB). Other unexplored sites of production of certain behavioral chemicals could be in resin exudates of the host tree or in the initial gallery constructed by attacking insects. As microorganisms, including fungi, are normal associates of insect guts, mycangia (in certain species), and galleries of bark beetles, and as they may contribute significantly to the total mass of fecal material, it is reasonable to assume that various frass associated pheromones occur either because of, or in spite of, the microorganisms. One can therefore, speculate whether a part of the pheromonal regime that attracts other insects for mating, mass attack, parasitation and predation could be of microbial origin.

Such a symbiotic relationship concerning the alteration of ingested or *in situ* host components seems a particularly plausible hypothesis for insects such as bark beetles so closely associated with microorganisms in their specialized diet and gallery system. Many species in the genera *Ips* and *Dendroctonus* are known to excrete the two hydroxylated derivatives of α-pinene, namely *cis*- and *trans*-verbenol, and, depending upon the species, either of these derivatives can exhibit pheromonal activity. The presence of various yeasts in the gut of *I. paraconfusus* has been established (Shifrine and Phaff 1956, Callaham and Shifrine 1960) and the fact that *d*-α-pinene can be transformed into both *d-cis*-verbenol and *d*-verbenone by a fungus was demonstrated by Bhattacharyya and coworkers (Bhattacharyya *et al.* 1960 and Prema and Bhattacharyya 1962).

One of us (JMB) has suggested that the pheromonal regime of bark beetles can arise, at least in part, from the microbial transformation of host-plant substances. These transformations could occur in the gut, in galleries in the phloem while under the influence of attacking beetles and associated microorganisms, and in frass after excretion. To test the hypothesis, microorganisms from the gut of both sexes of *I. paraconfusus* were isolated and their ability to convert *d*-α-pinene to *cis*- and *trans*-verbenol was assayed by gas chromatography and mass spectrometry. A strain of *Bacillus cereus* which produces *cis*- and *trans*-verbenol, and myrtenol from α-pinene has been isolated consistantly from these beetles. A *Bacillus* sp. from *I. grandicollis*,

D. frontalis, *D. brevicomis*, and *D. terebrans* that is capable of the same transformations has also been isolated. These three substances are known to occur in the gut of many bark beetles (Hughes 1973) and preliminary results by JMB with other microorganisms indicate that still other compounds of importance in bark beetle or predator behavior (*Thanasimus dubius*), such as verbonone can be made by different organisms associated with the beetles.

OBJECTIVES AND SCOPE

The object of this cooperative study is to investigate the production by microorganisms of a number of compounds known (or thought) to elicite behavorial responses in *D. frontalis* and/or important parasites and predators. It seems plausible that extracts of fermentations of the right organisms on the right substrates, possibly together with known compounds may be particularly effective in field traps. Such traps could be employed for various purposes. It might be possible to draw even larger numbers of bark beetle predators or parasites to areas of beetle infestations with appropriate blends of compounds. Chemical analyses of any extracts that might prove particularly effective could elucidate additional behavioral chemicals that influence beetle aggregation. The amount of material required for analysis is not likely to be a problem as large quantities of organisms can be grown at minimal cost.

This investigation will provide information not only useful for southern pine beetle population management, but will open the way for development of similar knowledge on other destructive bark beetles and field crop insects responding to pheromonal regimes.

METHODS

Microorganisms

Fungi, yeasts and bacteria used in this cooperative study will be provided through previous and on-going research by the Southern Forest Experiment Station, Pineville, La. (Barras and Perry 1972, Barras and Marler 1974a 1974b). These organisms include two fungi and two yeasts from the SPB mycangium, isolates of bacteria from beetle guts and egg galleries, and on an as yet undetermined number of yeasts from the galleries. Additional microorganisms will be isolated from resin of host trees under attack. Maintenance of the organisms before being subjected to pine chemicals will be on standard culture media. Whole pine phloem will be collected and shipped in dry ice to Iowa as needed in the study.

Transformation Experiments

The ability of all isolated microorganisms to transform or synthesize a variety of substrates (e.g. α-pinene, β-pinene, myrcene, *trans*-verbenol, verbenone, myrtenol, frontalin) will be studied. This will be done by, (1) growing organisms on a medium containing a substrate, (2) adding the substrate after growth has occurred, (3) adding the substrate to either whole cell suspensions or cell-free preparations, (4) growing organisms on phloem.

Chemical Analyses

Transformation products will be extracted with appropriate organic solvents and concentrated extracts will be subjected to gas chromatographic separation on a variety of columns. Purified compounds will be obtained from the extracts by preparative gas chromatography and mass spectra of isolated compounds will be compared to standards. When necessary, IR, NMR, and UV spectra will also be obtained.

Field Bioassay

Resulting chemical products showing promise as behavioral chemicals will be bioassayed alone or in combination with other known behavioral chemicals in traps designed by Moser (1975). The initial purpose of trapping will be to determine which of the several unknown compounds that may be produced by the microorganisms is attractive to SPB or parasites and predators. Replicate trapping procedures will be employed and the individual compounds or mixtures will be randomized among trap locations. Trapping will be done near an active SPB infestation to ensure a supply of beetles and associates.

FACILITIES

The Forest Insect Research Project (RWU 2203) possesses the necessary equipment for collecting, culturing and shipment of microbes to be used in the study. An active cooperative agreement with Louisiana State University-Alexandria (Agreement #19-178) will provide additional bacteria from SPB galleries.

The University of Iowa, Department of Microbiology, possesses equipment for the cultivation of microorganisms, the Infra-Red analysis and the gas chromatographic separation of compounds. Mass spectral analyses will be carried out on instruments in other departments on the campus (e.g. Chemistry, Pharmacology, Pharmacy) which are available to us. An NMR spectrometer, with Fourier transform, is available in the Chemistry Department.

Bioassay traps and trapping will be provided by the Forest Service. Additional assistance may be provided through proposed work period by JMB at the Pineville laboratory.

PRESENTATION OF RESULTS

Semi-annual reports and a final report will be prepared as required in the cooperative agreement.

COOPERATION

A formal agreement between the University of Iowa was executed on _____. It is expected that cooperation with LSU-A in the identification of bacteria from the SPB galleries will continue.

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